REMARKS

Claim rejection-35 U.S.C. § 112

Claims 1-10, 26-33, and 35 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner states:

> Claim 1, uses the term "selection marker" and "selection agent", it is not clear if the "selection agent" [is] an antibiotic (claim 10 or claim 32) or 5-AZA-C (claim 33).

PTO Paper dated 08/27/2003 at p. 3.

Applicants have amended claim 1 to more clearly recite "selection marker gene" and "selection drug".

Claim 2 has been amended to indicate that the selection is accomplished by antibiotic resistance. Moreover, Applicants disclose on page 22 of the specification that the helper virus is designed to contain a "marker selection gene". Such selectable marker may contain an antibiotic resistance gene.

Further, claim 33 has been amended to show that he demethylating agent 5-Aza-Cytidine can be used in addition to antibiotic selection. Support is found, for example, on page 8, 1st para. lines 1-4; page 43, line 24. In view of the amendments made, Applicants respectfully request this rejection be withdrawn.

The Examiner states:

> Claim[s] 2 and 27, it is not clear what is intended with "selecting helper virus which is functional" does this mean antibiotic selection or is another selection intended?

Id.

Although not acceding to the Examiner's rejection, Applicants have cancelled claim 3 and merged claim 3 into claim 2 to more clearly indicate that selection is by antibiotic selection.

Additionally, the claims have been amended to indicate that antibiotic selection is for cells having the functional helper virus, and not for the helper virus itself as it might have previously been interpreted. Furthermore, Applicants have amended claim 4 to depend from claim 2. Claim 27 has been amended to more clearly recite that selection is by antibiotic selection. In view of the amendments made, Applicants respectfully request this rejection be withdrawn.

The Examiner states:

> Claim 28, it is not clear what is intended with "removing from a population of vector packaging cells..." does this mean antibiotic selection or is another removal intended.

Id.

Claim 28 has been amended to recite "removing from a population of vector packaging cells, helper virus with 5' long terminal repeat methylation by introducing an antibiotic to said cells", to more clearly recite that removal is by antibiotic selection. Applicants respectfully request this rejection be withdrawn.

The Examiner states:

> Claim 29, it is not clear what is intended with "removing cell with inactivated virus by positive selection" does this mean antibiotic selection or is another selection intened?

Id.

Claim 29 has been amended to recite "the method of claim 28 further comprising the step of removing cells with inactivated virus by killing said cells with antibiotic selection", to more clearly recite that selection is by antibiotic selection. Claim 29 indicates that removal of cells with inactivated helper virus is by negative selection. Antibiotic resistant cells is positive selection. Applicants respectfully request this rejection be withdrawn.

The Examiner states:

> Claim 33, it is not clear if antibiotics selection in addition to treatment of cells with 5-AZA-C is intended or does this treatment alone selection for "functional helper virus."

Id.

Applicants have amended claim 33 to more clearly state that inhibition of methylation can also be achieved by treating vector producer cells with 5-AZA-C as an additional step of the method in combination with antibiotic selection as recited in claim 27, from which claim 33 depends. Support is found on page 43, lines 23-26 of the specification. Applicants respectfully request this rejection be withdrawn.

The Examiner states in part:

> The instant claims, specifically claim[s] 1 and 26, are indefinite in the recitation of a method for establishing vector packaging cells or "method of increasing the presence of viral titer" because the endpoint(s) of claimed methods are ambiguous and unclear. There is a lack of clarity as to critical or resolution steps or endpoints which reads back on the preamble of the claimed methods. Clarification so that the resolution step reads back on the preamble is required.

PTO Paper dated 08/27/2003 at p. 4.

Applicants have amended claims 1 and 26 to clarify the claims so that the resolution step reads back on the preamble. Applicants respectfully request this rejection be withdrawn.

The Examiner next states that the trademark Zeocin should not be used in claims 10 and 32.

Applicants have amended claims 10 and 32 to recite Phleomycin D1, a product commonly known as ZEOCIN™, thereby making the claim definite. (See MPEP 608.01(v)). Applicants respectfully submit that the recitation of Phleomycin D1, does not add new matter. Applicants respectfully request this rejection be withdrawn.

Claim rejection-35 U.S.C. § 102

Claims 1-4, 6-9, 26-31 and 35 were rejected under 35 U.S.C. § 102(e) being anticipated by Beach et al. (US Pat. No. 6,025,192 and US Pat. No. 6,255,071). The Examiner states in part:

> Beach et al disclose the production of a retroviral vector packing cell line (see section 5.6 US Pat. No. 6,025,192 col. 9-11 or US Pat. No. 6,255,071, col. 17-21). The reference discloses the production of a packing cell that provides gag, pol and/or env sequences which are then linked to an IRES linked to a selection marker in this case hygromycin (see Fig. 18 of US Pat. No. 6,255,071). Therefore, the instant invention is anticipated by Beach et al.

Id. at 5-6.

Applicants respectfully submit that US Pat. No. 6,025,192 and US Pat. No. 6,255,071 do not anticipate as they fail disclose transducing the packaging cell line with a viral vector comprising a packaging sequence and a gene of interest as required or states in claim independent claim 1 (See Applicants' claim 1) and decreasing the amount of inactive helper virus present in the vector packaging cell by providing for the selection of non-methylated helper virus-containing cells as stated in independent claim claim 26.

Additionally, Applicants have amended independent claims 1 and 26 to recite "a method for improving viral vector titer using a helper virus in a vector packaging cell line" and "a method for increasing the viral titer produced by a vector packaging cell upon transfection with a viral vector comprising decreasing the amount of inactive helper virus present in said vector packaging cell by selecting against DNA methlylation of helper virus, wherein said selection removes cells with methylated helper virus, thereby improving helper virus gene expression and virion production within a selected population of vector packaging cells", respectively, which provides for helper virus mediated vector producer cells. Claims 2-4, 27-31, and 35 contain by virtue of their dependency all the limitation of amended independent claims 1 and 26.

Moreover, Applicants respectfully submit US Pat. No. 6,025,192 and US Pat. No. 6,255,071 do not anticipate as the patents fails to disclose retroviral packaging cells lines made by using a helper vector that expresses virion components in coordination with the expression of a selectable marker. The helper packaging vectors disclosed by Beach et al. (Figs 17 and Figs 18 in US Pat. No. 6,255,071) base the high levels of expression of virion components (gag/pol and env) on the amplification of the vector plasmid copy number inside the cell's nucleus by providing an episomal origin of replication from Bovine Papilloma Virus (BPV) and by coexpression of the proteins (BPV-E1 and BPV-E2) necessary to amplify the vector copy number. As episomal vectors have a different chromatin rearrangement and lack essential chromosome scaffold proteins necessary for cytosine DNA methylation they are not subjected to DNA methylation in mammalian cells. Therefore, Beach et al. does not use drug selection to select for unmethylated chromosomal copies of helper vector, and does not teach how to avoid methylation of helper vector chromosomal copies.

This is in contrast to Applicants' method which uses drug selection in vector producer cells to eliminate cells bearing methylated chromosomal copies of helper vector in order to obtain a population of cells having non-methylated virus, which results in improved viral titer (e.g., see amended claim 1 and claim 5).

Applicants' have previously reported that DNA methylation could occur in the long terminal repeat region of helper virus in vector producer cells. To overcome host DNA methylation that suppresses viral gene expression, Applicants' have constructed a retroviral helper virus wherein the construction of the helper virus permits selection for intact and functional helper virus (see claim 2). The selection methods disclosed and claimed by the

Applicants' results in no DNA methylation of the helper virus, resulting in high helper virus expression and in high vector titers (e.g., see claim 2 and claim 27).

The helper vector system described by Beach et al. generates packaging cell lines that yield 15-fold less titer ($1x10^6$ cfu/ml) than the vector packaging cell lines disclosed in the Applicant's invention, which can yield titers of $1.5x10^7$ cfu/ml (see claim 5). These differences are attributable to different reasons which are mainly based on the episomal state and high vector copy number (between 100-1000 copies per cell) of the helper vectors disclosed by Beach et al. In the first place, gene expression from episomal vectors follows a different RNA processing and export pathway than chromosomal based gene expression. In general, episomal viruses have their own RNA control sequences to achieve efficient RNA processing and transport from the nucleus to the cytoplasm (i.e., tripartite RNA leaders of adenovirus) [Bridge et al, "Spliced exons of adenovirus late RNAs colocalize with snRNP in a specific nuclear domain" J Cell Biol. 1996 Oct;135(2):303-14; Bridge et al. "Nuclear organization of splicing small nuclear ribonucleoproteins in adenovirus-infected cells", J Virol. 1993 Oct;67(10):5792-802; Jimenez-Garcia et al. "In vivo evidence that transcription and splicing are coordinated by a recruiting mechanism"; Cell. 1993 Apr 9;73(1):47-59; Melcak I, "Nuclear pre-mRNA compartmentalization: trafficking of released transcripts to splicing factor reservoirs", Mol Biol Cell. 2000 Feb;11(2):497-510; Adami G et al "DNA template effect on RNA splicing: two copies of the same gene in the same nucleus are processed differently", EMBO J. 1991 Nov;10(11):3457-65]. The vectors disclosed by Beach et al do not have these sequences and therefore the RNA export and processing might be impaired. Second, having 100-1000 copies of episomal helper virus may result in sequestration and saturation of cellular factors such as transcription factors, RNA export factors, RNA splicing factors, cellular proteins involved in

protein folding, capsid assembly, etc., which are necessary for virion capsid synthesis and assembly. Third, the expression of viral capsid components is not coordinated with the expression of the vector genomic RNA that is packaged within those capsids. Having a very high expression of virion protein components that cannot be matched by stoichiometrically high levels of vector genomic RNA expression results in the production of empty capsids, devoid of vector genomic RNA. Upon transduction, this results in lower infectious rates (i.e., titers) due to neutralization of virus cellular receptors in the target cells by the empty capsids. The cellular receptor required for virus entry are competed out by the over-numbered empty capsid, which prevents the further infection (entry) of the same virus species or others as long as they use the same receptor for the infection. This phenomenon is known as (viral) Envelop protein-(cellular) receptor interference. Therefore, inclusion of episomal origin of replication and proteins that ensure high copy number of helper vector units within the nucleus actually has a negative effect on vector titers and people skilled in the art would not look into Beach et al to obtain stable packaging cell lines of defined and stable compositions if faced with the challenge of obtaining high titer vector preparations. It is not possible to obtain a packaging cell line of such characteristics by using cells containing high number of episomal copies of helper virus as the copy number in each cell within this population will fluctuate and change after every cell division. Moreover, high copy number of episomal vectors are highly recombinogenic, and therefore unstable.

This in is contrast to the Applicant's invention which generates a packaging cell line with unique or very low copy number of stably integrated chromosomal copies of the helper vector and the transfer vector. The cells generated by the applicants' method can be characterized, and their stability assessed by simple tests. Moreover, chromosomally integrated copies of the helper

vector are several orders of magnitude less likely to generate replication competent retrovirus by homologous or non-homologous DNA recombination. These characteristics make these cell lines applicable to manufacture retroviral vector preparations for clinical gene therapy applications.

Applicants respectfully submit that in view of the amendments made the claims and the arguments set forth above, US Pat. No. 6,025,192 and US Pat. No. 6,255,071 do not anticipate. Applicants respectfully request reconsideration and withdrawal of this rejection.

Claim rejection-35 U.S.C. § 103

> Claims 1-10, 26-31 and 35 were rejected under 35 U.S.C. § 103(a) being obvious in view of Beach et al. (US Pat. No. 6,025,192) or (US Pat. No. 6,255,071).

Id. at 7-8.

Applicants respectfully submit the claimed invention as a whole is not obvious to one of ordinary skill in the art. The invention as a whole is not only restricted to the specific subject matter claimed, but also embraces its properties and the problem it solves. Applicants' invention as claimed provides for vector packaging with improved efficiency by reducing the presence of inactivated helper virus. Ligation of IRES sequence followed by Zeo at the 3' end of the env gene permits the translation of helper virus open reading frame and a selection marker. Therefore, selection with Zeocin eliminates cells with methylated helper virus 5' LTR from the population. (See spec. p. 23). This design ensures sustained helper virus gene expression which would increase virion production. (See spec. p. 23, lines 21-24). Moreover, this is done in the absence of subcloning. (See spec. at p. 30, lines 5-22). On the other hand, Beach et al., US Patent Nos. 6,025,192 and 6,255,071 teaches methods that include the identification and isolation and nucleic acid molecules which complement a mammalian cellular phenotype, antisense-based

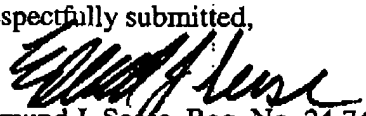
methods for the identification and isolation of nucleic acid sequences which inhibit the function of a mammalian gene, and gene trapping methods for the identification and isolation of mammalian genes which are modulated in response to specific stiumi. These patents do not teach or suggest a method for improving viral vector titer using a chimeric helper-virus-mediated vector packaging cell line. Absent such a suggestion, one of skill in the art would not look to these patents if confronted with the same problem as the Applicants (i.e., improving viral vector titer using a chimeric helper virus-mediated vector packaging cell line (claim 1) and a method for increasing the presence of viral titer produced by a vector packaging cell upon transfection with a viral vector (claim 26) in clinical applications. Therefore, claims 1-10, 26-31, and 35 are not obvious in view of Beach et al., US Patent Nos. 6,025,192 and 6,255,071. Applicants respectfully request this rejection be withdrawn.

Conclusion

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



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